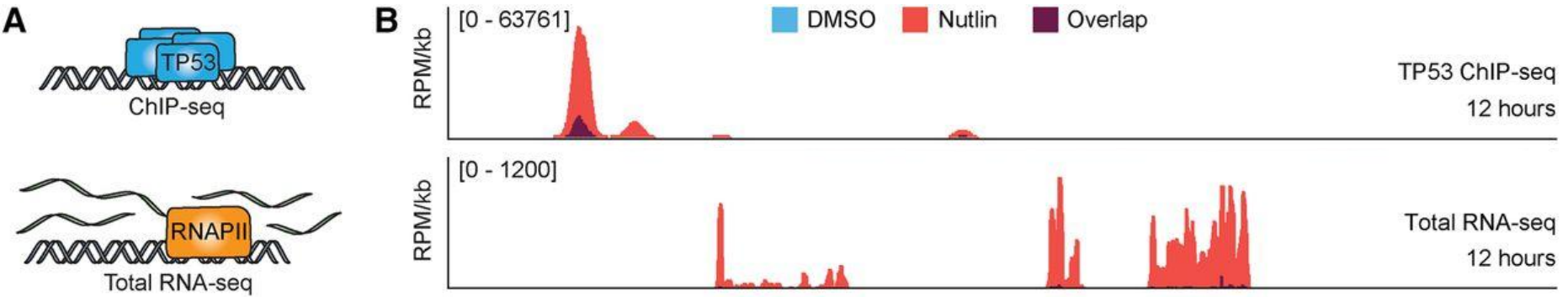


# Short Read Workshop Day 7

## *Counting Reads and Differential Expression*

Sam Hunter and Rutendo Sigauke  
2025

# Project B: Identification of the p53 transcriptional program using RNA-seq and ChIP-seq



Andrysik et al., 2017, doi: [10.1101/gr.220533.117](https://doi.org/10.1101/gr.220533.117)

In HCT116, SJSA, and MCF7 cell types.

Question: Which genes are driven by p53 activation?

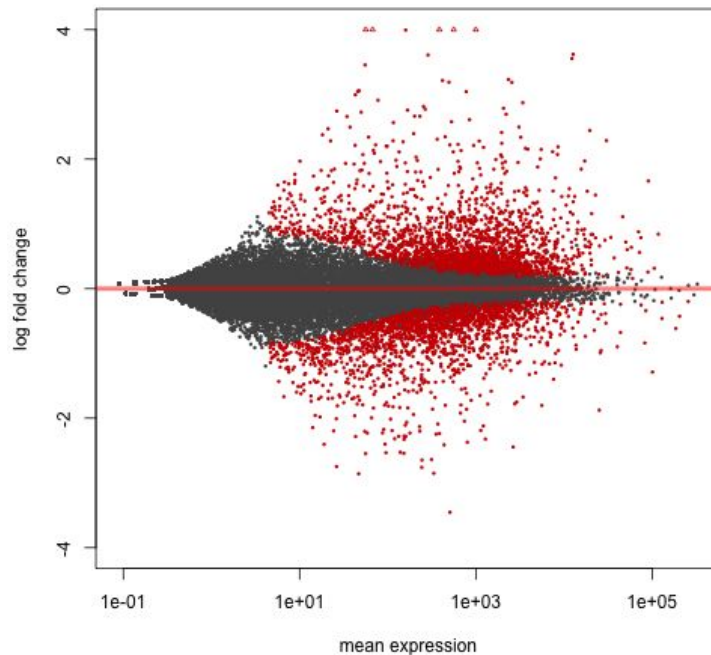
# Goal of the Day

Find genes that are different between samples

Map reads to reference genome

Count reads

Perform differential gene  
expression analysis



# featureCounts counts reads over features in R

There are several options in featureCounts

```
fc <- featureCounts(files=bam_file_list,
  annot.ext=gtf,
  isGTFAnnotationFile=TRUE,
  GTF.featureType="exon",
  GTF.attrType="gene_id",
  useMetaFeatures=TRUE,
  allowMultiOverlap=TRUE,
  largestOverlap=TRUE,
  countMultiMappingReads=TRUE,
  isPairedEnd=TRUE,
  strandSpecific=1,
  nthreads=N)
```

	union	intersection_strict	intersection_nonempty
	gene_A	gene_A	gene_A
	gene_A	no_feature	gene_A
	gene_A	no_feature	gene_A
	gene_A	gene_A	gene_A
	gene_A	gene_A	gene_A
	ambiguous (both genes with --nonunique all)	gene_A	gene_A
	ambiguous (both genes with --nonunique all)		
	alignment_not_unique (both genes with --nonunique all)		

aligned read:  
start: 113217600 end: 113217650



GTF

chr1	unknown	exon	113217048	113217252	-	+	gene_id "MOV10";p_id "P5535";transcript_id "NM_001130079"
chr1	unknown	exon	113217048	113217351	-	+	gene_id "MOV10";p_id "P5535";transcript_id "NM_020963"
chr1	unknown	exon	113217470	113217671	-	+	gene_id "MOV10";p_id "P5535";transcript_id "NM_001130079"
chr1	unknown	CDS	113217535	113217671	-	+	gene_id "MOV10";p_id "P5535";transcript_id "NM_001130079"
chr1	unknown	start_codon	113217535	113217537	-	+	gene_id "MOV10";p_id "P5535";transcript_id "NM_001130079"

↑  
feature type

↑  
feature

# Counting reads with featureCounts

- Follow [featureCounts](#) worksheet:
  - Open R and install Rsubread on AWS
  - Get `d7_featureCounts.R` and `d7_featureCounts.sbatch` scripts
  - Edit both scripts and execute the sbatch script

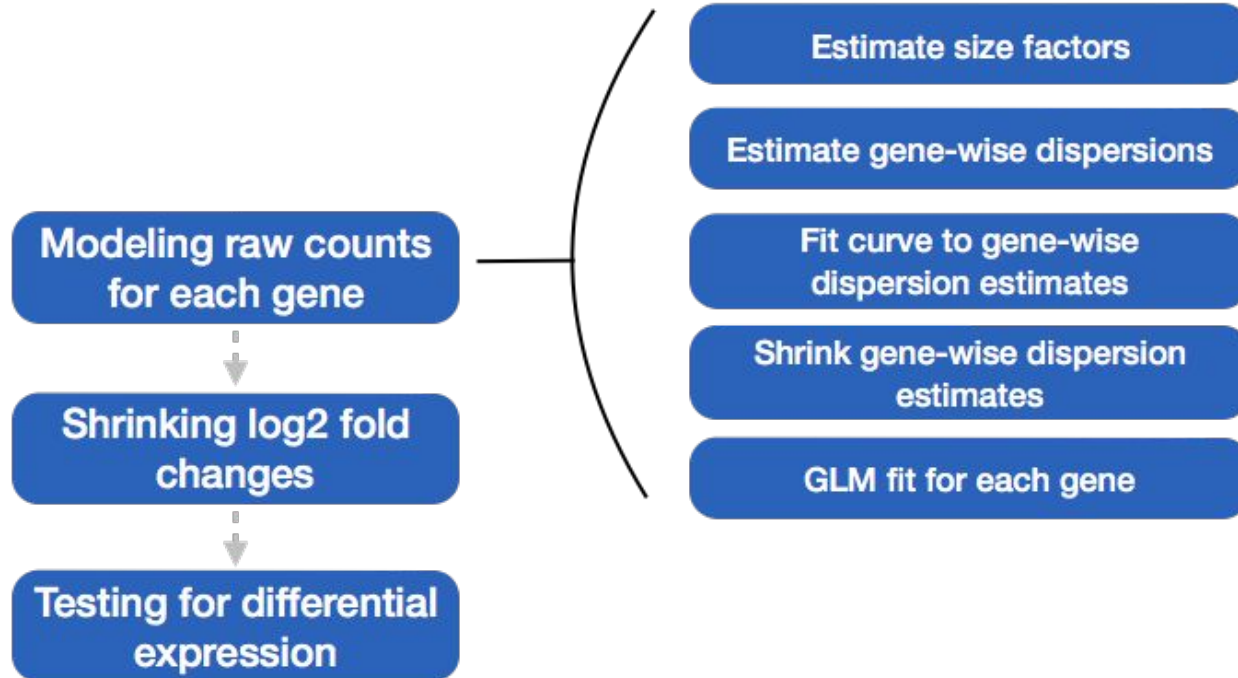
## Challenge Question

- What feature would you use to count reads for RNA-seq?
- A. Gene
  - B. Exon
  - C. Transcripts

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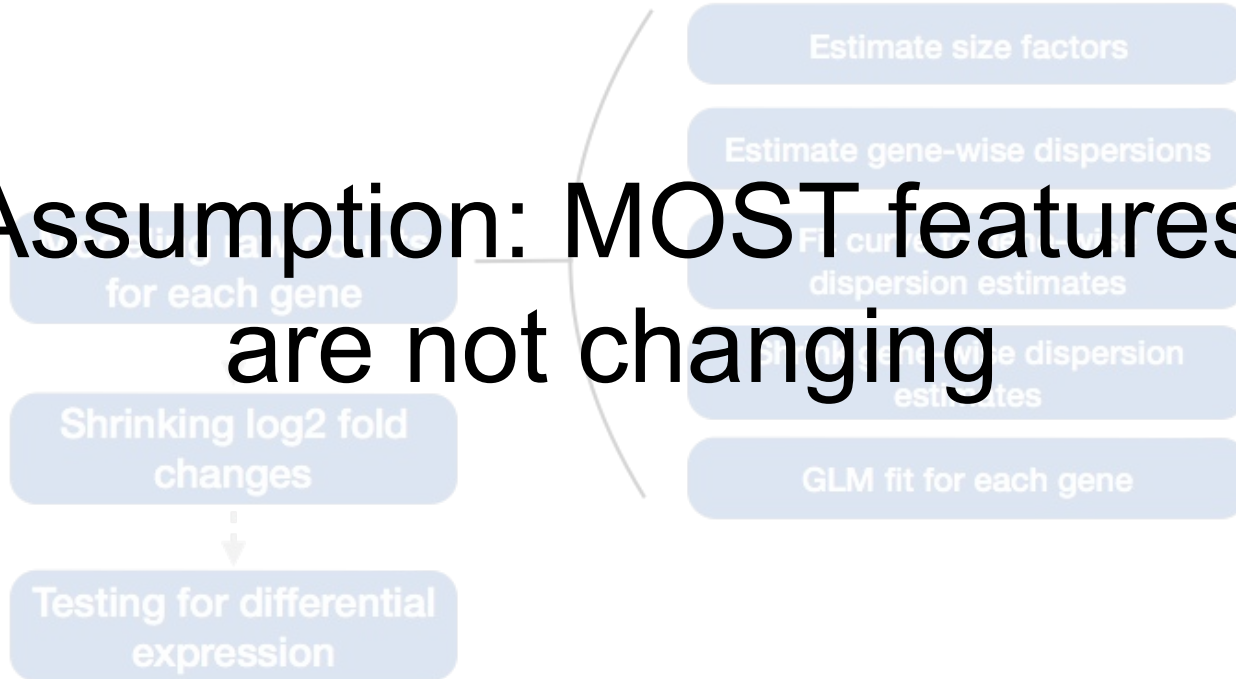
# DESeq2 Recap





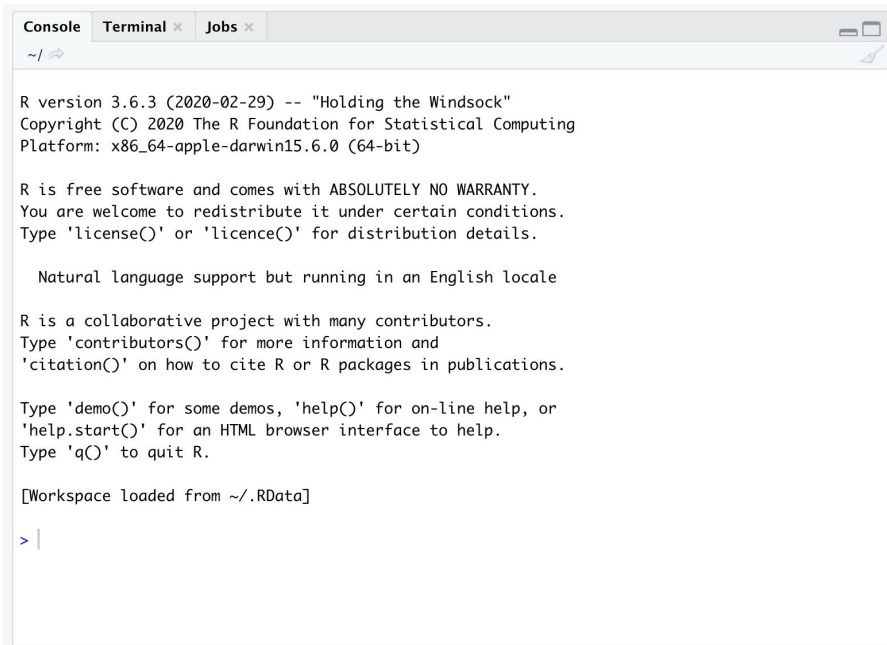
# DESeq2 Recap

Assumption: MOST features  
are not changing



# Run DESeq2...

- Follow [DESeq2](#) worksheet
  - This will be run in an R console in RStudio



```
Console Terminal x Jobs x
~/
R version 3.6.3 (2020-02-29) -- "Holding the Windsock"
Copyright (C) 2020 The R Foundation for Statistical Computing
Platform: x86_64-apple-darwin15.6.0 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

Natural language support but running in an English locale

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Workspace loaded from ~/.RData]

> |
```

# Challenge Question

- How would you run DESeq2 on the supercomputer?

# Challenge Question

- How would you run DESeq2 on the supercomputer?
  - Install DESeq2 in your R packages directory
  - Make a conditions table that matches your count table
  - Run the R script through an sbatch script

# Homework

- Run DESeq2 to explore differential expression with a different cell line